CURRENT METHODS FOR THE DIAGNOSIS OF PERTUSSIS INFECTIONS

Speakers

Lucia C. Pawloski, Ph.D, Biologist, Pertussis and Diphtheria Laboratory, Meningitis and Vaccine Preventable Diseases Branch, Division of Bacterial Diseases, National Center for Immunization and Respiratory Diseases Centers for Disease Control and Prevention, Atlanta, GA

Dr. Pawloski obtained her PhD in Genetics from the University of Georgia. She began her work at CDC as an ORISE fellow in the Pertussis and Diphtheria Laboratory in 2005 to assist in the development of serological diagnostic assays. Since then, she has collaborated with FDA to develop and evaluate the IgG Anti-PT ELISA kit that has proven to be a very useful diagnostic tool for pertussis in public health laboratories, surveillance, and outbreak response. Dr. Pawloski published the analytical validation of the IgG Anti-PT ELISA kit and co-authored several other publications on pertussis and diphtheria in peer-reviewed journals.

Lauren G. Pittenger, PhD, MBA, Senior Consultant, Booz Allen Hamilton, Atlanta, GA

Dr. Pittenger has worked on a range of projects including laboratory capacity modeling for the influenza group, production and procurement support for the Laboratory Response Branch, Project managementfor the Bacterial Rapid Response and Advanced Technology laboratory, and QMS implementations for several groups at CDC.

She is an adjunct Professor of Microbiology at Georgia Perimeter College.

Previously, she worked as Laboratory Manager/Grad Student for the USDA- ARS, in Athens GA. While at USDA she developed a microarray for studying Campylobacter jejuni, developed DNA sequencing capabilities for the research station, and managed the lab.

Kathleen M. Tatti, PhD, Biologist**,** Pertussis and Diphtheria Laboratory**,** Meningitis and Vaccine Preventable Disease Branch**,** Division of Bacterial Diseases**,** National Center for Immunization and Respiratory Diseases Centers for Disease Control and Prevention, Atlanta, GA

Dr. Tatti has over twenty-five years of experience in bacteriology, virology and molecular biology. In 2005, she joined the Pertussis and Diphtheria Laboratory (PDL). As a member of PDL at CDC, she has worked on molecular diagnostics of pertussis and developed a multiplex PCR assay for the diagnosis of pertussis. She has authored and co-authored over 40 peer-reviewed manuscripts, reports and book chapters, several of which involve the laboratory diagnosis of pertussis.

Objectives

At the conclusion of this program, participants will be able to:

- Discuss current testing methods for pertussis diagnosis: real-time PCR, serology and culture.
- Describe the two new assays developed by CDC and how they could add value to your current testing algorithm.
- Develop a greater understanding for the need to move towards standardized testing.

Continuing education credit is no longer available for this program

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Current Methods for Diagnosis of Pertussis Infections

Pertussis and Diphtheria Laboratory
Meningitis and Vaccine Preventable Diseases Branch
Division of Bacterial Diseases
National Center for Immunization and Respiratory Diseases
Centers for Disease Control and Prevention

Atlanta, GA
January 21, 2010

Presented by

Kathleen Tatti
DBD, CDC
KTatti@cdc.gov

Lucia Pawloski
DBD, CDC
LPawloski@cdc.gov

Lauren G. Pittenger
Booz Allen Hamilton
pittenger_lauren@bah.com

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Objectives

- Understand current testing methods for pertussis diagnosis and implications in public health
  - Culture
  - Real-time PCR
  - Serology
- Become familiar with the two new assays developed by CDC and how they could add value to your current testing algorithm
  - Multiplex PCR
  - IgG anti-PT ELISA
- Realize the importance of complementary testing
- Develop a greater understanding for the need to move towards standardized testing

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Outline

- Background
- Epidemiology
- Diagnostics — **Lauren Pittenger**
  - Culture
  - Real-Time PCR — **Kathleen Tatti**
  - Serology
- Conclusions
- Future Directions — **Lucia Pawloski**

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Pertussis Background and Culture

Lauren Pittenger
pittenger_lauren@bah.com

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Pertussis Background

- Severe, debilitating cough illness (“100 day cough”)
- Highest morbidity and mortality rates in infants
- Despite high vaccine coverage, remains a public health problem
- Clinical diagnosis and laboratory confirmation can be challenging
- Outbreaks regularly occur

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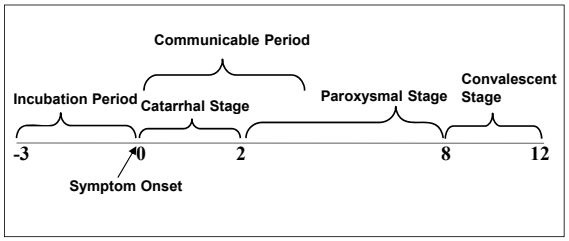
Stages of Disease in Weeks



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Pertussis CSTE Case Definition

- Clinical case definition
  - Cough ≥2 weeks and at least one symptom: paroxysms, whoop, posttussive vomiting
- Case classification
  - Confirmed cases
    - Culture positive
    - Clinical case and PCR positive
    - Clinical case and epi-linked to confirmed case
  - Probable case
    - Only meets the clinical case definition

* Serology not yet included in the case definition/classification

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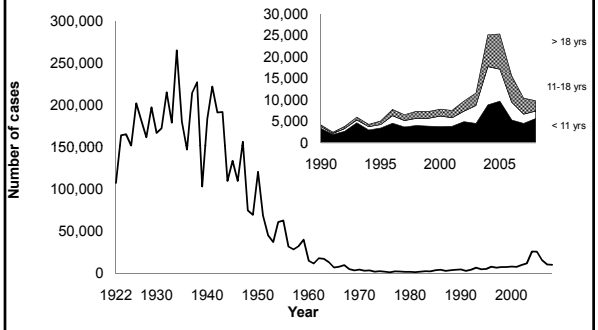
Reported Pertussis Cases

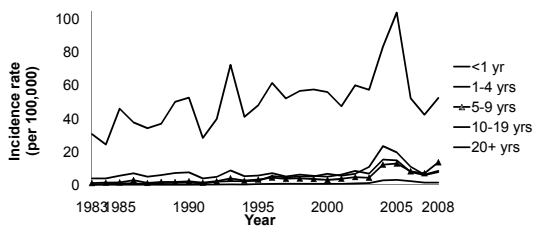
United States 1922-2008



•SOURCE: **CDC, National Notifiable Diseases Surveillance System and Supplemental Pertussis Surveillance System and** 1922-1949, passive reports to the Public Health Service

Handout

Reported Pertussis Incidence by Age Group, US 1983-2008



Source: CDC, National Notifiable Diseases Surveillance System and Supplemental Pertussis Surveillance System and passive reports to the Public Health Service

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Diagnostic Needs
Clinical vs. Public Health

- Clinical setting
  - Optimizes sensitivity
  - Rapid turnover
- Public health setting
  - Optimizes specificity
  - Confirmation of etiology
  - Prevention and control measures

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Outbreaks that Exhibit the Need for Complementary Testing

- Hospital: Winter 2006
- School: Winter 2007
- Community: Summer 2009

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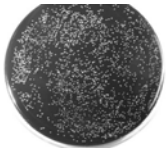
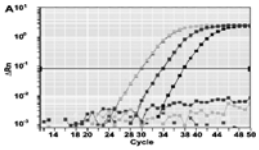
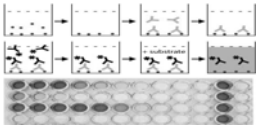
Pertussis Diagnostics

- **Culture**
- **Real-Time PCR (R-PCR)**
- **Serology**







Handout

Diagnostic Tests for Pertussis

Test	Sensitivity	Specificity	Optimal Timing	Advantages	Disadvantages
Culture	12 – 60%	~ 100%	< 2 weeks post-cough onset	Very specific (100%)	Low sensitivity; 7-10 day delay between specimen collection and diagnosis
PCR	70 – 99%	86 – 100%	< 4 weeks post-cough onset	Rapid test; more sensitive than culture; organisms do not need to be viable; positive post-antibiotics	No FDA approved tests or standardization; potential for false positives; DNA contamination is problematic
Paired* Sera	90 – 92%	72 - 100%	At symptom onset and 4-6 weeks later	Effective indication of mounting antibody titers	Late diagnosis; no FDA approved tests or standardization
Single* Sera	36 - 76%	99%	At least 2 weeks post-cough onset; ideally >4 weeks post-cough	Useful for late diagnosis post antibiotics	No FDA approved test or standardization; possibly confounded by recent vaccination; diagnostic cut-offs not validated

*Not part of CDC/CSTE case definition (Exception: MA single point ELISA assay)
** Sensitivity and specificity values obtained from Wendelboe and Van Rie, 2006.

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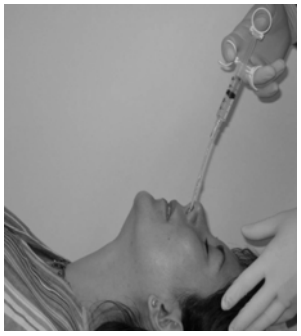
Specimen Collection



- Specimen type will impact ability to isolate bacterium
  - Nasopharyngeal (NP) aspirates yield similar or higher rates of recovery than NP swabs (rayon or polyester)
  - Throat and anterior nasal swabs yield unacceptably low rates of recovery

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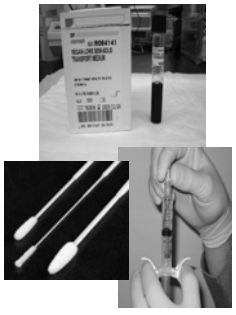
After Specimen Collection

- Plate immediately or place into Regan-Lowe transport medium
- Dispensing and plating should be completed within 24 hours of specimen collection
- Specimen can be used for both culture and PCR



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Culture

- "Gold Standard"
  - Essential for public health labs
- 100% specific, but low sensitivity
  - Most sensitive within first two weeks after cough onset
- Highest yield
  - Young patients
  - Unvaccinated patients
  - Patients early in cough illness prior to antimicrobials
- Incubation time 4-10 days
- Although specific collection methods, transport, media and growth conditions are needed, culture is not difficult

Gram stain of *B. pertussis*

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Methods for Culturing Pertussis

- Regan-Lowe or Bordet-Gengou media
- Inoculate on media with and without antibiotics
- 35-36°C incubation with high humidity
- Ensure plates do not dry out
  - Plastic bags
  - Canisters
  - Pan of water
- Check plates every day

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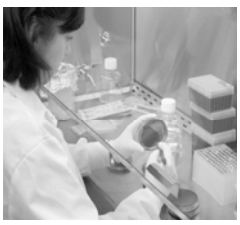
Growth Characteristics

- Bordet-Gengou (BG)
  - Small colony size
  - Appearance similar to mercury droplets
  - Colonies appear hemolytic
- Regan-Lowe (RL)
  - Small colony size
  - Glistening, cut glass appearance



Bordetella on RL Medium

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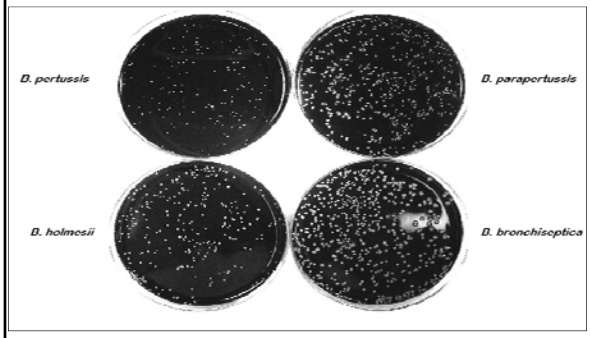

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Other *Bordetella spp.*

- *B. parapertussis*
  - Colonies typically appear within two-three days
  - On RL agar the colonies will appear greyish
  - On BG agar colonies have a brown pigmentation
- *B. holmesii*
  - Colonies look similar to *B. pertussis*
  - Growth is inhibited by cephalexin
- *B. bronchiseptica*
  - Large colonies
  - Appear after one day
  - On RL agar the colonies will have a slight brown coloration

Public Health Impact of Pertussis Culture

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- Particularly important if an outbreak is suspected
- Isolation of the bacterium confirms pertussis
  - Other respiratory pathogens often cause similar clinical symptoms
  - Co-infection with other pathogens does occur
  - Colony morphology helps to identify other species of *Bordetella*
- Necessary for antimicrobial susceptibility testing and molecular typing

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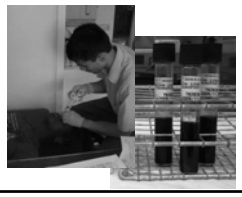
Culture is Feasible

School Outbreak 2007

- Proper transport medium allowed excellent recovery of *B. pertussis* when direct plating could not be performed
  - Culture was performed at CDC
  - Overnight shipment was not available



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Pertussis Real-Time PCR

Kathleen Tatti
KTatti@cdc.gov

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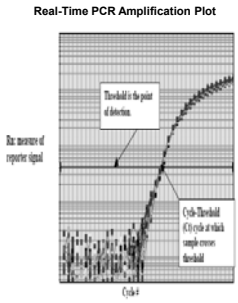
R-PCR Assay-IS*481*

- Present in three *Bordetella* species
  - 50 to >200 copies in *B. pertussis*
  - 8 to 10 copies in *B. holmesii*
  - One copy in *B. bronchiseptica* (host specific)
- High Ct value could indicate
  - Positive test result
  - False positive
  - Positive result of a *Bordetella* species other than *B. pertussis*



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Available online at www.sciencedirect.com
ScienceDirect
Diagnostic Microbiology and Infectious Disease 61 (2008) 264–272
DIAGNOSTIC MICROBIOLOGY AND INFECTIOUS DISEASE
www.elsevier.com/locate/diagmicrobio

Development and evaluation of dual-target real-time polymerase chain reaction assays to detect *Bordetella* spp.☆

Kathleen M. Tatti*,[1], Kai-Hui Wu[1], Maria Lucia Tondella, Pamela K. Cassiday, Margaret M. Cortese, Patricia P. Wilkins, Gary N. Sanden

Meningitis and Vaccine Preventable Diseases Branch, Division of Bacterial Diseases, National Center for Immunization and Respiratory Diseases, Centers for Disease Control and Prevention, Atlanta, GA 30333, USA

Received 1 November 2007; accepted 27 February 2008

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MMWR
Morbidity and Mortality Weekly Report

Weekly August 24, 2007 / Vol. 56 / No. 33

Outbreaks of Respiratory Illness Mistakenly Attributed to Pertussis — New Hampshire, Massachusetts, and Tennessee, 2004–2006

http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5633a1.htm

Handout

MMWR Article

- Outbreaks were attributed to pertussis based only on local single-target PCR (IS*481* assay)
- 2 specimens were positive for *B. holmesii* by PCR
- Only one specimen was positive by CDC two-target assay
- PCR results were not confirmed by the two-target assay at CDC
- Limitations of relying solely on a single-target PCR to confirm pertussis outbreaks
- Emphasizes importance of
  - Diagnostics validation
  - PCR result interpretation
  - Standardization of PCR assays

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Benefit of Dual Target- Hospital Outbreak 2006

AB 7000 N=111	*ptxS1*+	*ptxS1*-
IS*481*: Ct<35	**1**	**2**
IS*481*: 35≤Ct<40	**0**	**22**
IS*481* -	**0**	**86**

MMWR, Outbreaks of respiratory illness mistakenly attributed to pertussis, 2004-2006. 2007;56:837-842

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Decontamination

- Throughout this process
  - Keep your lab coat on and wear gloves
- Four step process
  - 10% bleach
  - 70% ethanol
  - DNA AWAY ®
  - UV exposure for a minimum of one hour

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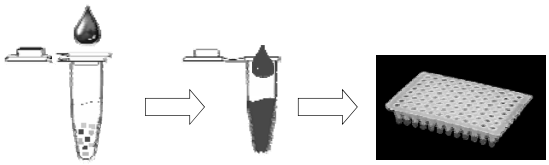
Multiplex R-PCR Assay for *Bordetella* spp.

- **IS*481* primers/probe set FAM- *B.pertussis*/*B. holmesii***
- **hIS*1001* primers/probe set Quasar- *B. holmesii***
- **pIS*1001* primers/probe set HEX- *B. parapertussis***



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Multi-target R-PCR Allows for Speciation

Species	*ptxS*1	Multiplex		
		IS*481*	hIS*1001*	pIS*1001*
B. pertussis	+	+	-	-
B. parapertussis	+	-	-	+
B. pertussis* and *B. parapertussis	+	+	-	+
B. holmesii	-	+	+	-

Handout

Multi-target R-PCR Approach

- The multiplex assay utilizes three target sequences
  - IS*481*
  - hIS*1001* specific for *B. holmesii* (3-5 copies/cell)
  - pIS*1001* targets *B. parapertussis* (20-23 copies/cell)
- The *ptxS*1 targets the gene for the S*1* subunit of pertussis toxin
  - Single copy in *B. pertussis* and *B. parapertussis*
- Additional assay targets human *rnase*P

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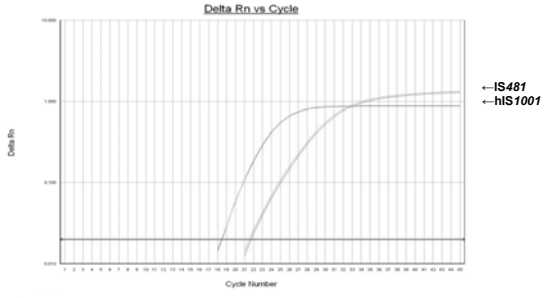
Clinical Specimen with *B. holmesii*

Hospital Outbreak 2006



Clinical Specimen with *B. pertussis* and *B. parapertussis*

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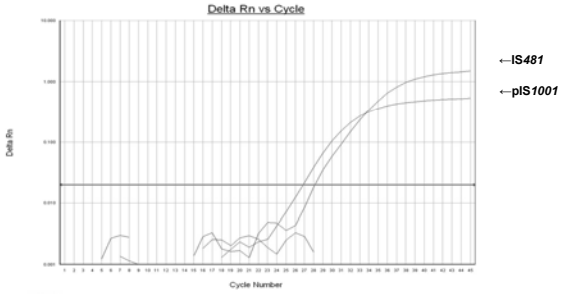

Analytical Sensitivity and Clinical Relevance

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Genomic equivalents	*Bordetella pertussis ptxS*1 Ct values	*Bordetella pertussis* IS*481* Ct values
1000	29	19
100	32	23
10	35	26
1	39	30
0.1	**Negative**	33

Analytical Sensitivity and Clinical Relevance

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Genomic equivalents	*Bordetella pertussis ptxS*1 Ct values	*Bordetella pertussis* IS*481* Ct values	*Bordetella parapertussis* IS*1001* Ct values	*Bordetella holmesii* hIS*1001* Ct values
1000	29	19	22	25
100	32	23	26	29
10	35	26	30	33
1	39	30	33	38
0.1	**Negative**	33	36	**Negative**

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CDC R-PCR Pertussis Outbreak Algorithm

	IS*481*+ (Ct<35)	IS*481*+ (35≤Ct<40)	IS*481*- (Ct≥40)
***ptxS1*+ (Ct<40)**	*B. pertussis*	*B. pertussis*	*B. parapertussis*[1]
***ptxS1*- (Ct≥40)**	*B. holmesii*[2]	Indeterminate	Negative

[1] Confirmed by pIS*1001* target

[2] Confirmed by hIS*1001* target

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R-PCR Interpretation Criteria

- Interpret R-PCR results along with the clinical symptoms and epidemiological information
- Determine the clinically relevant cut-off value for all targets
- Use indeterminate as a result for multi-copy target for IS*481,* not *ptx*S1

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Whooping Challenges

Respiratory Outbreak Investigation in Colorado, U.S.

CDC investigates mysterious pertussis cases in Durango

August 20th, 2009, 8:25 am · Post a Comment · posted by Brian Newsome

A team from the U.S. Centers for Disease Control and Prevention has flown from Atlanta to Durango to investigate a spike in pertussis cases with unusual symptoms, according to the health department there.

The San Juan Basin Health Department say
whooping cough, in La Plata County since
even though the Southwest Colorado region

Many of the cases are mild and don't have th
health department says. The unusually high
disease prompted the Colorado Departmen

The four-person team is interviewing people
find a cause.

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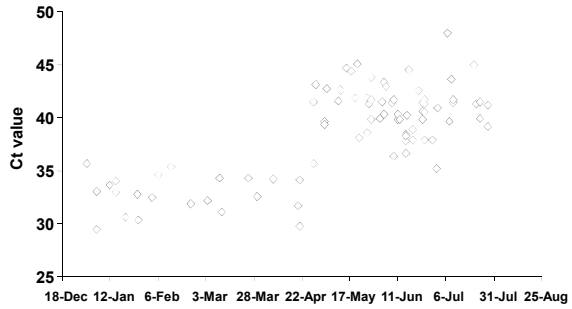
Importance of Cut-Off Values
Community Outbreak 2009



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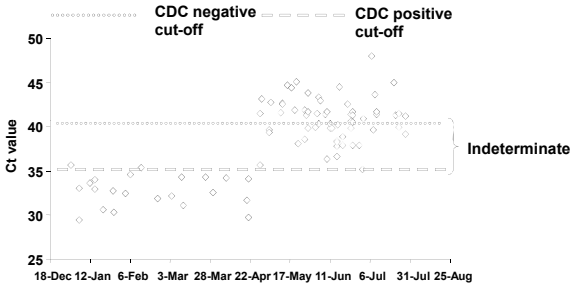
Importance of Cut-Off Values
Community Outbreak 2009



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Addressing the Myths of a Multi-target Approach

Concern	Resolution
• Will reduce sensitivity	• Data has shown that there is no reduction in sensitivity
• Multiplex is more expensive	• Initial cost may be higher • Fewer re-tests
• It will take longer to generate data	• Assay does not take longer to run than a single target assay
• It is a technically difficult assay to perform	• No more difficult than a single target PCR
• New equipment will have to be purchased	• Maybe, some older platforms can't accommodate multiplex

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Pertussis Serology

Lucia Pawloski
LPawloski@cdc.gov

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Pertussis Serology

- Originally designed for vaccine evaluation
- Routinely used for diagnosis in other countries
- Included as part of Massachusetts' case definition
- Useful for confirming diagnosis, especially during outbreaks where culture is not performed
- Can be used later in disease

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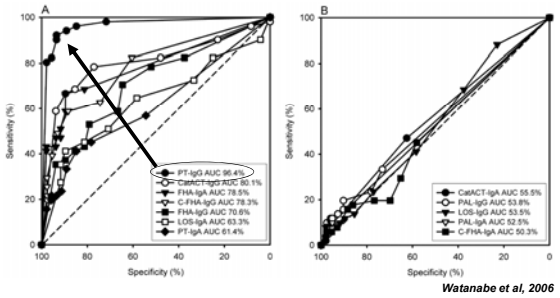
IgG Against Pertussis Toxin (PT) is the Most Specific and Sensitive Target



Watanabe et al, 2006

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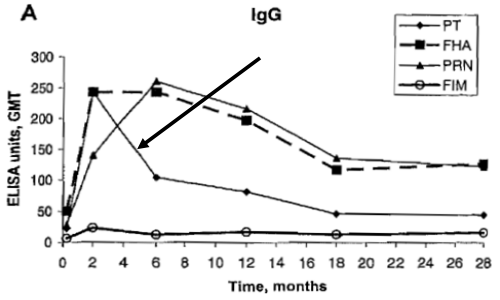
IgG Allows for Diagnosis of Recent Infections



Heininger et al. 2004

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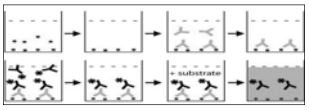
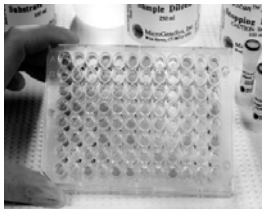
CDC/FDA IgG Anti-PT ELISA Kit

- If present, specific IgG antibodies in the serum will bind to the PT adsorbed to the wells
- The concentration of the PT IgG antibodies is directly proportional to the intensity of the color





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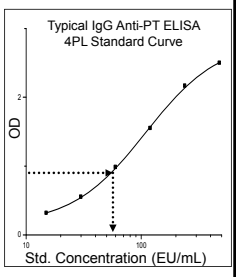
Easy to Use

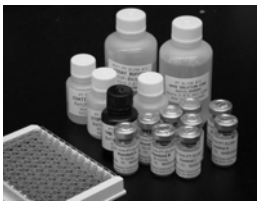
- 6 ready-to-use standards
- 3 ready-to-use controls
  - 49, 94 EU/mL and negative
- Standard curve can be generated
- Simple assay / production process
  - Single dilution of serum
  - Minimal reagent preparation
  - Incubation at room temperature
  - Calibrated to reference sera
  - Monoclonal antibody conjugate



Handout

IgG Anti-PT ELISA Kit Protocol

- Day 1
  - Coat PT onto microtiter wells
  - Incubate for 14-20 hours
- Day 2
  - Wash; add standards, controls, and test samples (1:100 diluted)
  - Incubate 2 hours at RT
  - Wash and add the conjugate antibody
  - Incubate 2 hours at RT
  - Wash and add the substrate
  - Incubate 10 minutes at RT
  - Add stop solution
  - Read at 450 nm



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CLINICAL AND VACCINE IMMUNOLOGY, Dec. 2009, p. 1781–1788 Vol. 16, No. 12
1556-6811/09/$12.00 doi:10.1128/CVI.00248-09

Development and Analytical Validation of an Immunoassay for Quantifying Serum Anti-Pertussis Toxin Antibodies Resulting from *Bordetella pertussis* Infection[▽]

Sandra L. Menzies,[1]* Vijay Kadwad,[1,4] Lucia C. Pawloski,[2] Tsai-Lien Lin,[3] Andrew L. Baughman,[2] Monte Martin,[2] Maria Lucia C. Tondella,[2] Bruce D. Meade,[1,5] and the Pertussis Assay Working Group

Division of Bacterial, Parasitic, and Allergenic Products, Center for Biologics Evaluation and Research, FDA, Rockville, Maryland[1]; Division of Bacterial Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia[2]; Division of Biostatistics, Center for Biologics Evaluation and Research, FDA, Rockville, Maryland[3]; Immunoassay Department, BRIT, Mumbai, India[4]; and Meade Biologics, LLC, Hillsborough, North Carolina[5]

Received 19 June 2009/Returned for modification 28 August 2009/Accepted 19 October 2009

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Addressing the Myths of Serodiagnosis

Concern	Resolution
• Recent vaccination may confound diagnosis	• Post-vaccination antibody levels do not interfere with diagnosis
• Paired sera is necessary	• Single serum point taken at the optimal time is sufficient
• Special analytical software is needed	• Assay can be qualitative
• It takes too long to perform	• Can be run in as few as 20hrs
• Publication of single strain lacking PT gene	• Isolates tested to date have PT gene

Public Health Use of Pertussis Serology

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Outbreak	Sera	Positive	Indeterminate
Hospital 2006	39	1	5
School 2007	169	49	10
Community 2009	15	0	0

Optimal Timing for Diagnostic Testing

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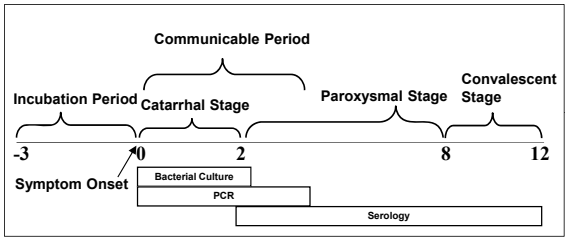

Test Results by Cough Duration

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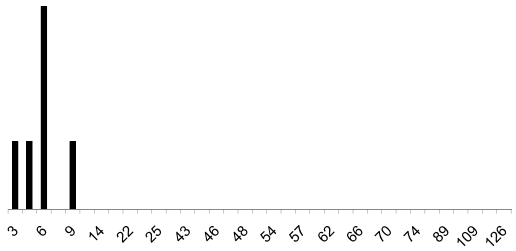
School Outbreak 2007



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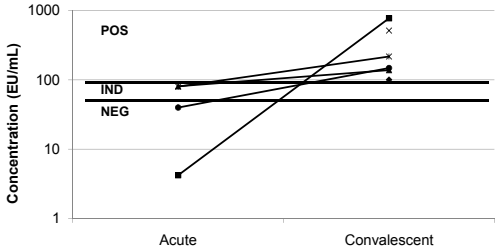
Timing Matters

6 culture and PCR positive patients seroconverted



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Conclusions

- No single laboratory test can stand alone for diagnosing pertussis
- Adoption of multi-target R-PCR methods will allow for confirmation and speciation among *Bordetella* spp
- Serology is a useful method for diagnosing pertussis especially in adults and in the later stages of the disease
- Labs should maintain culture capabilities
- Standardization is needed
- Diagnosis is an important part of surveillance

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CDC can Provide

- Protocols and draft SOPs for testing methods
- Culture
  - Support and advice on techniques
- PCR
  - Support with running on CDC validated PCR platforms: ABI 7500 and other platforms
  - Provide guidance on use of many extraction methods
- Serology
  - Support and advice on development, implementation, and standardization of ELISA

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Future Directions
Clinical Validation Study

- Estimate the clinical sensitivity, specificity and predictive values of diagnostic tests:
  - CDC/FDA IgG anti-PT ELISA
  - CDC's combined multi-target R-PCR
- Assess clinical usefulness as related to
  - Stage of disease, age, antibiotic use and vaccination status

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How can State and Local Labs Help?

- Help with early identification of outbreaks
  - Contact:
    Meningitis and Vaccine Preventable Diseases Branch
    404-639-3158
    Ask for the duty officer
- Send isolates to CDC
- Adopt standardized assays when available

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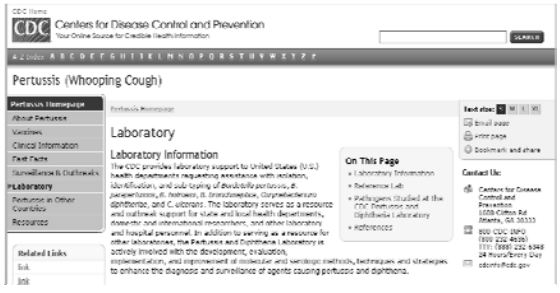
Pertussis Website



http://www.cdc.gov/pertussis/lab.html
(Under Development)

Diagnostic Testing is Important to Public Health

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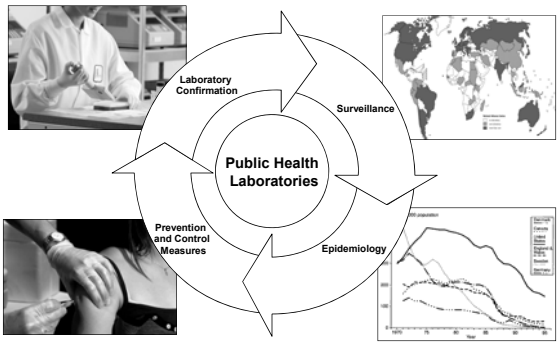

Acknowledgements

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- Pertussis and Diphtheria Lab
  - M. Lucia Tondella
  - Kathleen Tatti
  - Lucia Pawloski
  - Pam Cassiday
  - Kathryn O'Connell
  - Monte Martin
  - Michelle Bonkosky
  - Amber Schmidtke
  - Aditi Kapasi
- CDC/NCIRD
  - Wendi Kuhnert
  - Stephen Hadler
- CDC/MVPDB Epi Team
  - Nancy Messonnier
  - Tom Clark
  - Stacey Martin
  - Matthew Griffith
  - Sema Mandal
  - Amanda Faulkner
  - Alison Patti
- APHL
  - Kathleen Breckenridge
  - Travis Jobe
  - Rosemary Humes

Contacts

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Team Lead
M Lucia Tondella
MTondella@cdc.gov

Serology
Lucia Pawloski
LPawloski@cdc.gov

PCR
Kathy Tatti
KTatti@cdc.gov

Culture
Pam Cassiday
PCassiday@cdc.gov

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The findings and conclusions in this report have not been formally disseminated by the Centers for Disease Control and Prevention/the Agency for Toxic Substances and Disease Registry and should not be construed to represent any agency determination or policy.

Handout

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